Summary

In this review, the principles behind, and the utility of, fractionation of subcellular organelles by centrifugal techniques have been explored. Common methods used, and a review of the advantages and disadvantages of certain experimental tools (centrifuges, rotors, and aqueous media used for fractionation) were also reviewed and tables and graphs useful for designing protocols were also provided.

See also: **II/Centrifugation:** Large-Scale Centrifugation; Theory of Centrifugation.

Further Reading

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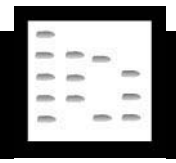
Field Flow Fractionation

DropBooks

See **III / CELLS AND CELL ORGANELLES: FIELD FLOW FRACTIONATION**

PAINTS AND COATINGS: PYROLYSIS: GAS CHROMATOGRAPHY



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Because they are complex polymeric materials, usually compounded with coloured or opaque fillers, paints and coatings, especially when dry, pose a difficult analytical problem. Gas chromatography in particular, and mass spectrometry, may seem unlikely tools for the analysis of such materials, but when combined with pyrolysis as a sample introduction technique, they may be used routinely. Via pyrolysis, the polymers used in paints and coatings are fragmented to produce molecules small enough to be compatible with gas chromatography–mass spectrometry (GC-MS), but still characteristic of the original material. Natural polymers, including plant resins and drying oils, as well as synthetic polymers like polyesters, acrylics and polyurethanes, have been studied extensively, and may be easily differentiated using this technique.

Pyrolysis

The general purpose of paints and coatings is to apply a protective or decorative film of material on to a substrate. The range of materials which may be used to form such a film is extremely wide, as is the range of techniques used to apply it. Soluble materials may be dissolved in a suitable solvent and applied as a thin

coat which produces a film as the solvent evaporates, as in the case of many varnishes and lacquers. On the other hand, materials may be applied which react in place, linking into a complex polymer network. Materials may even be applied as a powder which is subsequently melted into a film. The paint or coating may include many different constituents, both organic and inorganic, to give it the required physical and visual properties, including opacity, flexibility, colour, resistance to water, shine, durability and so on.

Once applied and set, paints and coatings have one characteristic in common: the molecules which comprise the finished product are almost always much too large to be analysed by GC. In fact, chemical analysis of dried paint is a difficult challenge by any technique, since the sample material is such a complex mixture of different polymers, additives, pigments and fillers. Analytical pyrolysis, however, permits the use of GC and MS in the investigation of these materials by breaking the large molecules into fragments small enough to be compatible with these instruments. Samples are typically heated rapidly to temperatures in the range of 600–800°C. At these temperatures, the organic macromolecules undergo bond dissociations based on the relative strengths of the bonds employed in the compound. Consequently, the molecule will fragment in a reproducible way, consistent with the chemistry of the compound, and form products which are directly indicative of the composition. This is true for simple homopolymers such as polystyrene, but also true for complex systems involving multiple monomers and various constituents. Frequently, the small molecules formed include the monomer or monomers of polymers, in addition to indicative products specific for various polymer types.

Instrumentation

The following sections discuss specific types of paints and coatings, with example pyrograms for many of these types. Unless indicated in the figure legends, all of the chromatograms were produced using the instrument parameters described here.

Pyrolysis

Samples were pyrolysed using a platinum coil filament autosampler (model 2500 autosampler, CDS Analytical Inc, Oxford, PA, USA). Approximately 100 μg of sample material was placed into a quartz tube and held in place using quartz wool. The samples were placed online with the GC carrier flow, and then heated to the indicated temperatures for the specified times, typically 750°C for 15 s.

Gas Chromatography–Mass Spectrometry

A model 6890 (Hewlett-Packard, Wilmington, DE) gas chromatograph was equipped with a HP-5 column (30 m long, 0.25 mm diameter, 0.25 μm film) operated with a 75 : 1 split ratio and helium as the carrier gas, in the constant pressure mode at 5.9 psi. The oven initial temperature was 40°C for 2 min, then programmed at 6°C min^{-1} to 300°C, held for 5 min. A mass selective detector (model 5972A, Hewlett-Packard, Wilmington, DE) provided the mass spectra. Scans were taken at a rate of 2.4 s^{-1} over the mass range of 35–550.

Paints and Varnishes

Varnishes

Clear, decorative or protective finishes are formulated and applied in a variety of ways. A varnish may be as simple as a plant resin (such as mastic, sandarac or dammar) or shellac (secreted by insects) dissolved in a solvent. A film of the solid organic material is left on the surface of the object varnished after the solvent evaporates. More likely, the formula includes drying oils – unsaturated fatty acids which polymerize when exposed to air – plus natural or synthetic resins, thinners and other additives. When pyrolysed, the dried varnish film creates indicative molecules from the various constituents, revealing much about its composition. The natural resin dammar, for example, is comprised extensively of triterpenes, made from three terpene units or six isoprene units. When pyrolysed, dammars generate chromatograms (pyrograms) showing groups of terpenes, as do the other plant resins used in making varnishes. **Figure 1** is a pyrogram of Kremmer dammar, which was heated to 650°C for 15 s. The peaks at about 25 min are sesquiterpenes, having three isoprene units, and the materials eluting at about 45 min are diterpenes (two terpene or four isoprene units). Other terpenoid resins used in making varnishes behave in a similar way, producing characteristic chromatograms permitting them to be differentiated. By contrast, when shellac is pyrolysed, it reveals a mixture of complex fatty acids, esters and olides, as shown in **Figure 2**.

Polyurethanes are formed from the reaction of a diisocyanate with a di- or polyfunctional alcohol. When pyrolysed, the most characteristic material formed is the regeneration of the diisocyanate. This is the case whether the polyurethane is a foam rubber-type polymer, or used as part of a paint or varnish. Hexane diisocyanate and toluene diisocyanate (TDI) are two commonly used polyurethane constituents, but others, both aromatic and aliphatic, are encountered as well. **Figure 3** is a pyrogram obtained by

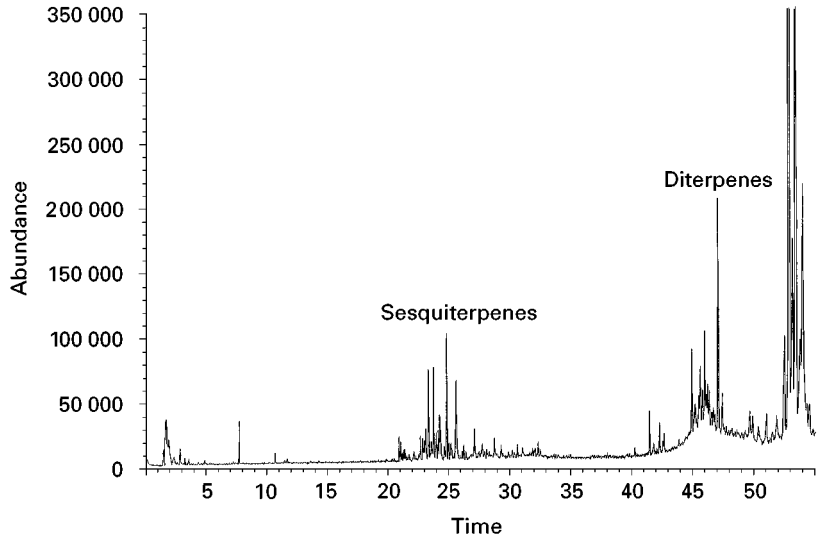

Figure 1 Kremmer dammar pyrolysed at 650°C for 15 s.

heating a sample of a polyurethane film to 700°C for 20 s, in which the large peak at 21 min is identified as TDI.

Oil finishes, based on large amounts of drying oils, are popular for furniture. Tung oil in particular is high in C_{18} polyunsaturated fatty acids, which polymerize when exposed to oxygen. Pyrolysis products from pure tung oil include some of the unsaturated acids, plus hydrocarbons, both aromatic and aliphatic, formed from the long chain of the acid. Commercial tung oil finish products are likely to contain other polymerizing agents as well. **Figure 4** shows the pyrogram of a recently dried film produced from a commercial tung oil product. The peaks at 32 and 36 are unsaturated fatty acids seen in pure tung oil, but there is also a peak at 21 min marked T, which is TDI, showing that this product incorporated polyurethane as well. Exterior waterproofing finishes may also incorporate drying oils, and other things as well. The pyrogram shown in **Figure 5**, from a wood waterproofing product, reveals both long chain fatty acids (for example, the largest peak at 37 min) as well as a series of hydrocarbons eluting from 38 to 45 min.

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House Paints

Alkyd Glyceryl phthalate polyesters have been used for many years to make paints for a wide range of uses, including interior and exterior housepaint. The reaction of the difunctional acid and the polyfunctional alcohol creates the polyester film. When pyrolysed, the ester linkage of the polyester is cleaved,

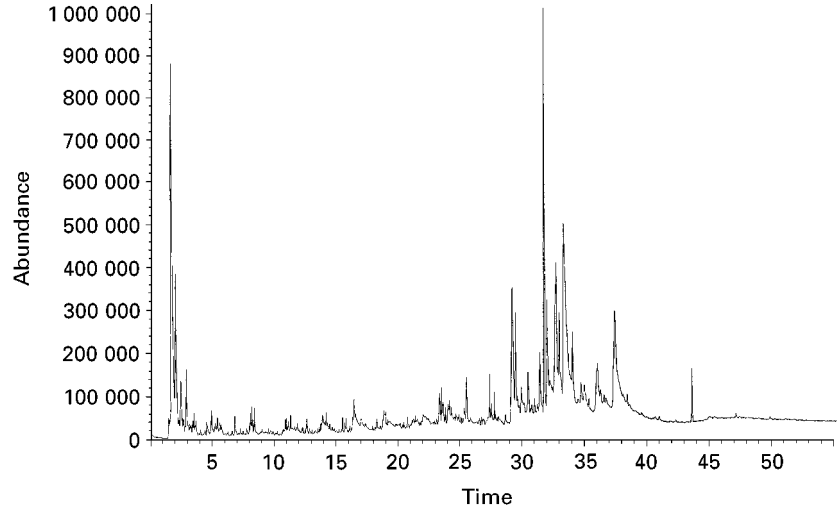

Figure 2 Pyrolysis of shellac at 700°C for 15 s.

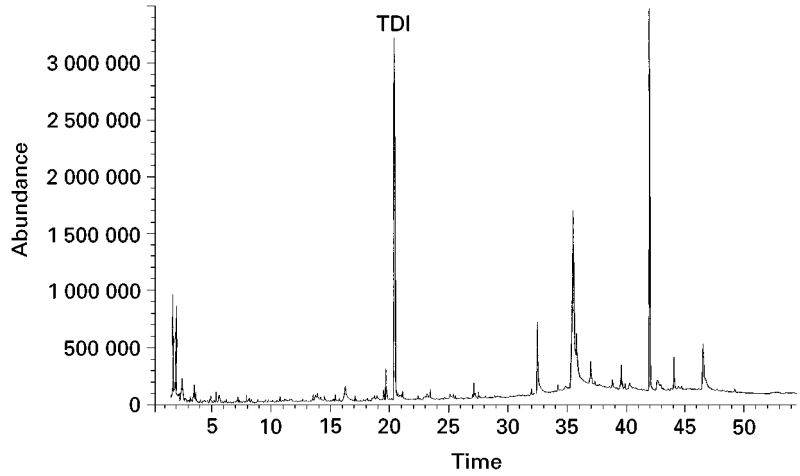

Figure 3 Pyrolysis of a polyurethane film at 750°C for 20 s. TDI at about 21 min is toluene diisocyanate.

resulting in the formation of phthalic anhydride, the characteristic pyrolysate for these paints. An example of this is shown in **Figure 6**, produced from a light green interior semi-gloss trim paint. The large peak at 20 min is phthalic anhydride, the most abundant pyrolysis product. Smaller constituents elute before this peak, mostly oxygenated degradation products from the alcohol portion of the polyester. In addition, peaks are generated from the drying oils in the formulation, in this case eluting at about 33 min (C_{16}) and a small peak at 36 min (C_{18}).

Latex Latex housepaints are substantially different. These water-based emulsions are almost always formulated using a variety of different polymers and copolymers, offering an essentially unlimited range of formulations. Typical polymers used include polyvinylacetate (PVA), polymethylmethacrylate (PMMA), polystyrene and polybutylacrylate (PBA) as well as higher acrylics. When pyrolysed, many of these polymers regenerate sufficient monomer to be identified in the pyrolysate. This is true for PMMA, polystyrene, PBA and other acrylates and especially for methacrylates. Acrylates, like PBA, and polystyrene also produce higher oligomers, especially the dimer and trimer. In cases of copolymers, there may be mixed dimers and trimers as well, if the different monomers are part of the same polymer chain. PVA, however, does not produce monomer. Because of the relative bond weakness, acetic acid is split from the

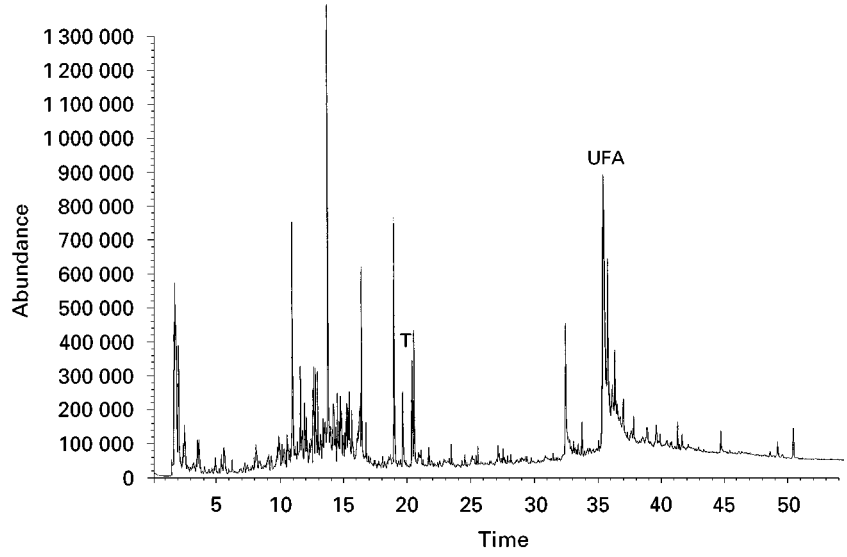

Figure 4 Pyrolysis of dried tung oil finish. Peak labelled T is toluene diisocyanate; UFA is a C_{18} unsaturated fatty acid.

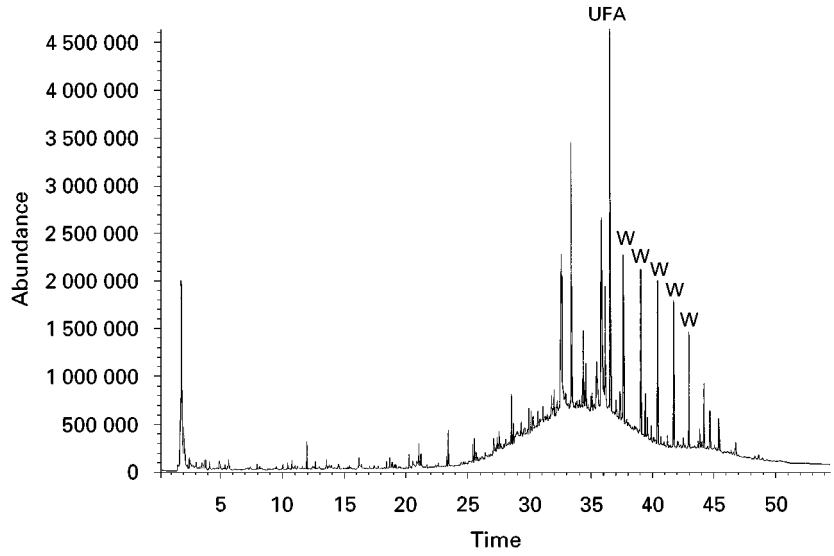

Figure 5 Pyrolysis of a waterproofing film at 750°C for 20 s. Peak marked UFA is a C_{18} unsaturated fatty acid. The peaks marked with a W are from a hydrocarbon wax.

polymer backbone before the chain is broken. As the acetic acid leaves the polymer, the backbone becomes highly unsaturated. Consequently, when the chain finally does break, it generates aromatics. Therefore, instead of vinyl acetate monomer, the pyrolysis products include acetic acid, benzene, toluene and other substituted aromatics.

The pyrogram of a flat green latex wall paint shown in **Figure 7** illustrates much of this. The paint is largely PVA, as shown by the large acetic acid peak eluting at about 3 min. Peak number 2 is one of the aromatics formed from the PVA chain (toluene). In addition, the paint included styrene and butyl acrylate, which elute at about 8 min. The white latex analysed in **Figure 8** is significantly different. For this paint, the largest peak is styrene, with the amounts of styrene and butyl acrylate both substantially increased relative to the PVA, and therefore dimers and trimers of these monomers may be seen eluting later in the chromatogram. In addition, there is a peak for methyl methacrylate as well.

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Spray paint The flat black, indoor/outdoor spray paint shown in **Figure 9** was deposited from a spray

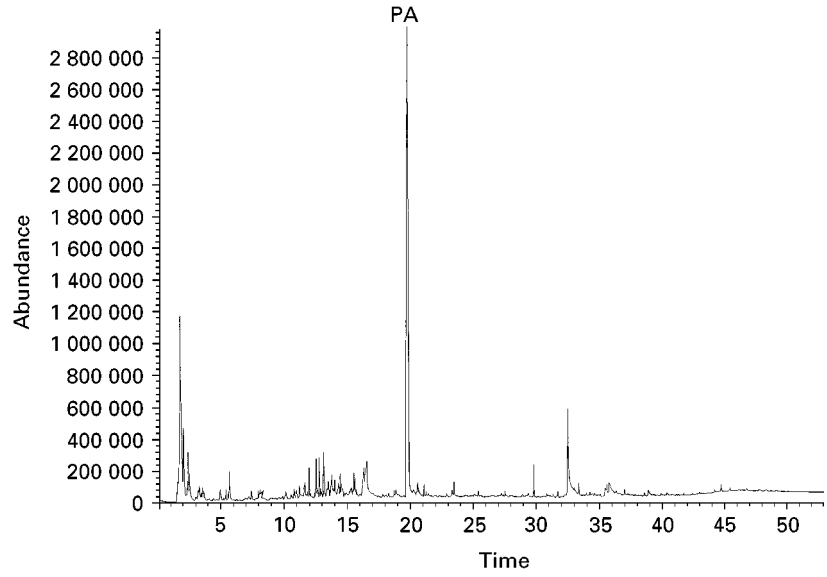

Figure 6 Pyrogram of a glyceryl phthalate alkyd housepaint. The peak labelled PA is phthalic anhydride.

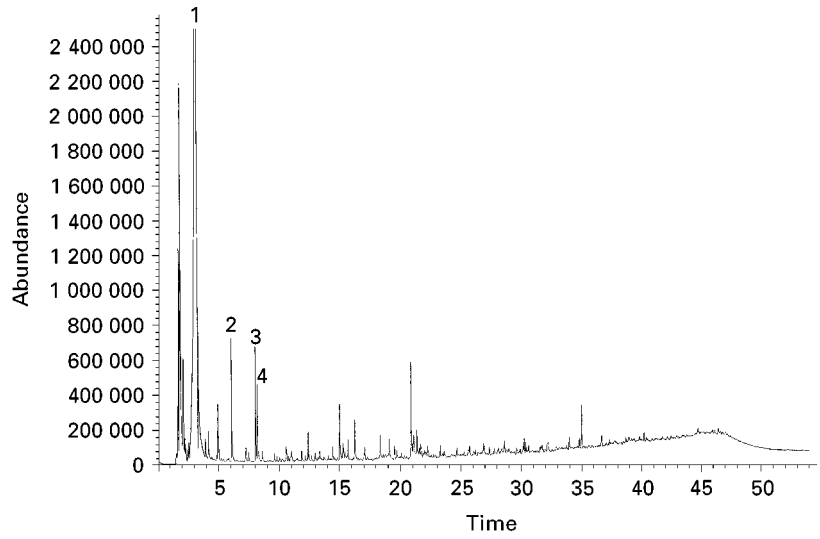

Figure 7 Pyrolysis of a latex paint at 750°C. 1, Acetic acid (from PVA); 2, toluene; 3, styrene; 4, butyl acrylate.

which contained acetone and xylenes. In fact, the peak at about 7 min is a xylene, probably retained in the paint matrix and released as the sample was heated. The pyrogram shows methyl methacrylate (4 min) phthalic anhydride (20 min) and a series of peaks between 32 and 37 min derived from drying oils, especially a C_{18} fatty acid.

Beverage container paint The paint pyrolysed to produce the chromatogram in **Figure 10** was scraped from an aluminium can which contained a carbonated soft drink. The paint was formulated using mostly methyl methacrylate, with some butyl acrylate and a little styrene.

In all of the paint examples here, only the major polymer constituents have been stressed. Paints, of course, contain many other ingredients, such as stabilizers, catalysts, solvents and additives, and peaks from some of these may appear in the chromatogram. In addition, there are pigment and materials to make the paint opaque. Many of these are inorganic, and although they may be present in large quantities (for example, TiO_2), their presence rarely affects the progress of the pyrolysis in creating identifiable fragments from the polymers.

Automotive paints Through the 1950s, automobile paints were likely to be glyceryl phthalate polyesters.

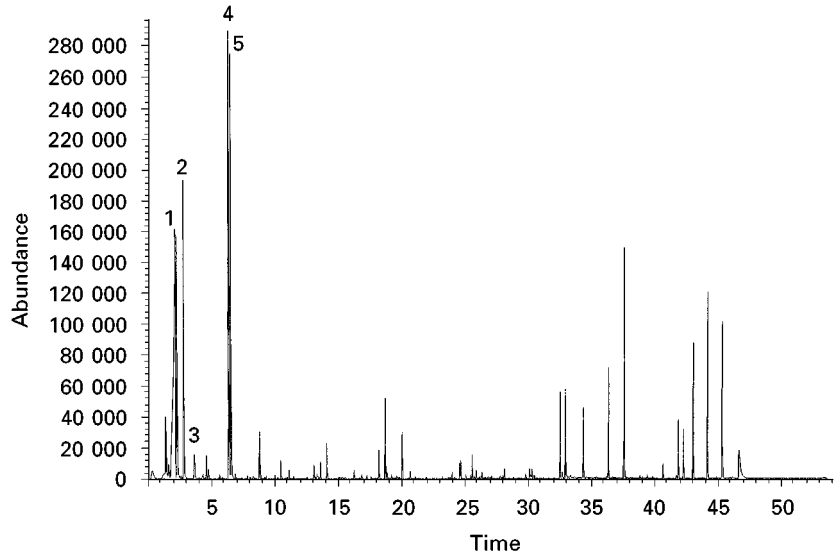

Figure 8 Pyrogram of latex paint at 700°C. 1, Acetic acid; 2, methyl methacrylate; 3, toluene; 4, styrene; 5, butyl acrylate.

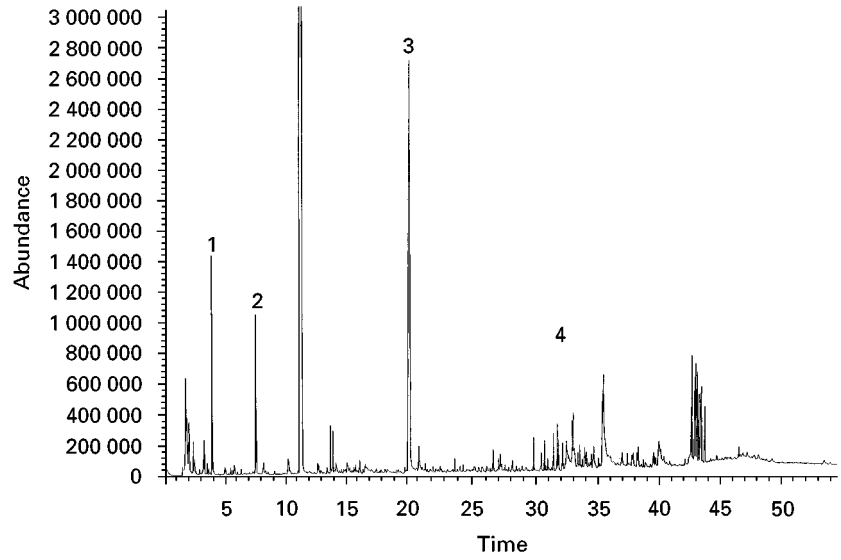

Figure 9 Pyrolysis of a flat black spray paint. 1, Methyl methacrylate; 2, xylene; 3, phthalic anhydride; 4, drying oils.

As described above, these phthalate polyesters pyrolyse to produce a characteristic peak for phthalic anhydride, plus other small molecules from the polyol. To control shrinkage and provide flexibility, plasticizers were added to these paints, generally in the form of a phthalate ester. When the paint sample is pyrolysed, it is typical to find that the plasticizer has been volatilized intact, and consequently it shows up in the pyrogram as well, even though strictly speaking it is a thermally desorbed molecule, not a pyrolysis product. This can be seen in the pyrogram shown in **Figure 11**. The paint is a black du Pont product used on a 1955 Chevrolet, and the sample pyrogram it produced has a large peak for phthalic anhydride at about 19 min, and peak for the plasticizer (dibutyl phthalate) at 31 min. In the 1960s these paints were replaced with other formulations, initially using mostly PMMA with a phthalate plasticizer. In the 1970s other monomers were added to the mix, including styrene, butyl acrylate and butyl methacrylate. Since polymers using these monomers produce significant amounts of monomer when pyrolysed, pyrograms of such an automotive finish look like the example in **Figure 12**, where peak 1 is methyl methacrylate, 2 is styrene, 3 is butyl acrylate, 4 is butyl methacrylate and 5 is butyl benzyl phthalate.

It must be pointed out that automotive finishes may be very complex systems, incorporating multiple

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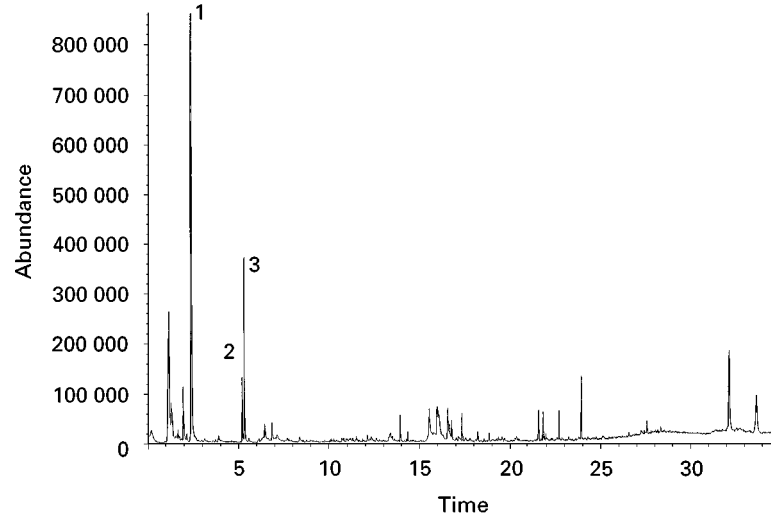

Figure 10 Paint from an aluminum soda can. 1, Methyl methacrylate; 2, styrene; 3, butyl acrylate. GC programme 40°C for 2 min, then 10°C min^{-1} to 300°C.

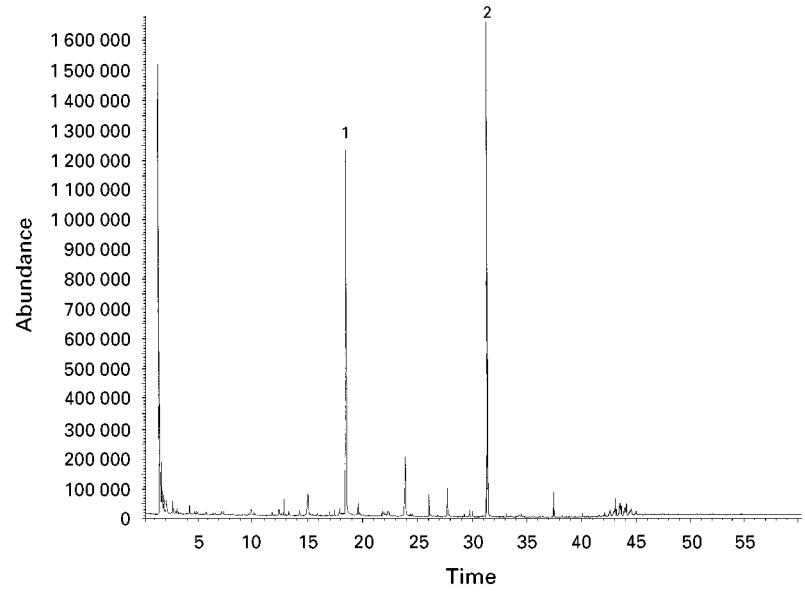

Figure 11 Paint from a 1955 black Chevrolet. 1, Phthalic anhydride; 2, dibutyl phthalate, a plasticizer.

layers, each of which is probably a different paint formulation. Further, these layers may be cross-linked with specific reagents, filled, coloured, etc. The examples shown here will deal primarily with the polymer composition of the paints, but information is frequently present concerning additives, especially plasticizers and light stabilizers, as well.

In an effort to reduce the amount of solvent vented to the air when a car is painted, manufacturers have developed paints with a higher solids load (and consequently less solvent per application). These paints are usually solutions of smaller polymer molecules, which are linked together during drying to form larger, cross-linked polymers. The standard monomers (methyl methacrylate, styrene, butyl acrylate, butyl methacrylate) are still used, but others are added to provide sites for cross-linking. These are frequently hydroxy compounds, and the OH functionality is used to form urethane, epoxy or other bonds during cross-linking. Hydroxyethyl methacrylate and hydroxypropyl methacrylates are used in the paint polymer chain to provide these sites. When paints incorporating the hydroxy methacrylates are pyrolysed, these monomers are produced just as the others are, and the pyrogram looks like

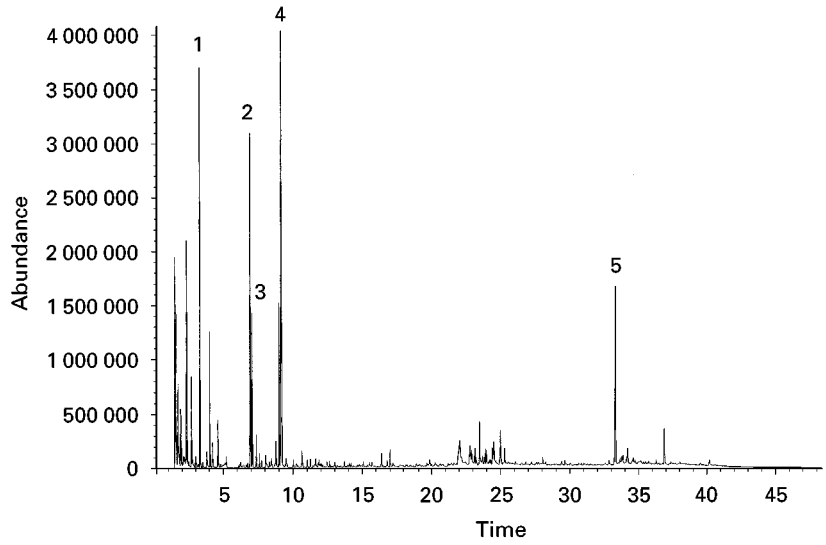

Figure 12 Pyrogram of automobile paint including multiple monomers. 1, Methyl methacrylate; 2, styrene; 3, butyl acrylate; 4, butyl methacrylate; 5, butyl benzyl phthalate (plasticizer).

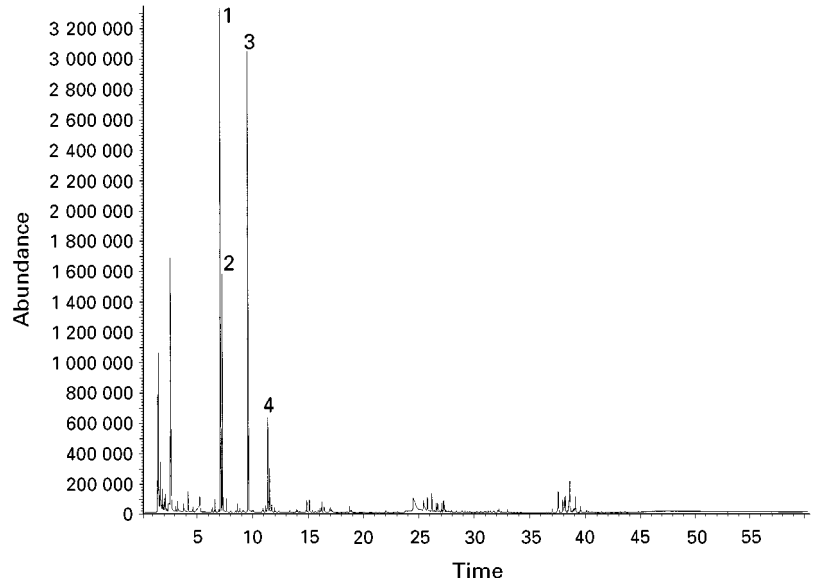

Figure 13 Pyrolysis of black acrylic enamel car finish. 1, Styrene; 2, butyl acrylate; 3, butyl methacrylate; 4, hydroxy propyl methacrylate.

Figure 13, in which styrene (peak 1), butyl acrylate (2) and butyl methacrylate (3) are major pyrolysis products. Peak 4 is 2-hydroxy propyl methacrylate, a common monomer added to provide cross-linking sites.

It is not unusual to see evidence of other polymers and polymer constituents in automotive finishes, either as the cross-linkers or from one of the many paint layers. Urethanes and epoxies are frequently used, and the characteristic compounds from these materials are frequently identifiable. The paint shown in **Figure 14** employed a urethane in addition to the monomers already discussed. When pyrolysed, it reveals methyl methacrylate, styrene, butyl acrylate and butyl methacrylate (peaks 1–4 in the pyrogram) and also shows TDI (peak 5 at about 17 min). Epoxies are frequently made using bisphenol A, which is regenerated on pyrolysis and provides a good indicator. The very complex pyrogram of a 1993 red car paint shown in **Figure 15** includes a peak at 31 min for bisphenol A, evidence of an epoxy formulation, in addition to standard monomers and a large peak of phthalic anhydride at about 17 min.

As a further step in improving the performance of automotive finishes, manufacturers are incorporating

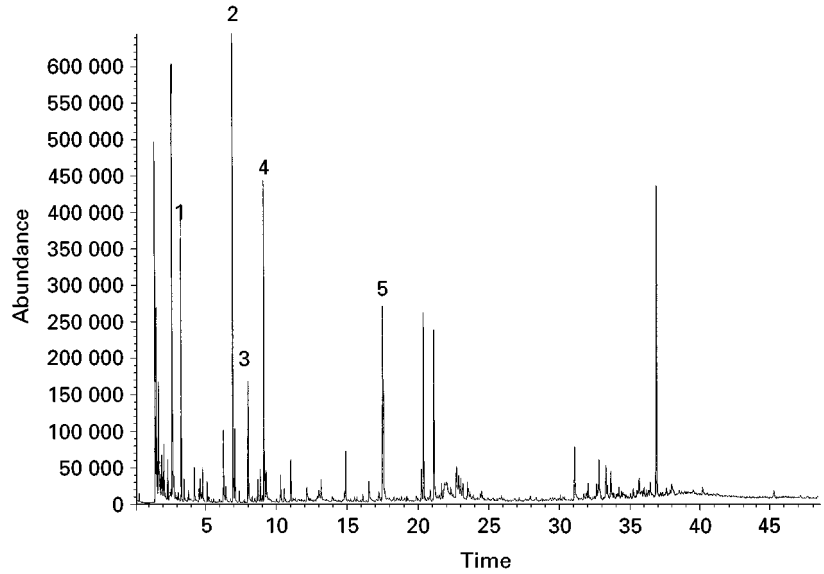

Figure 14 Pyrogram of automotive finish including urethane bonds. 1, Methyl methacrylate; 2, styrene; 3, butyl acrylate; 4, butyl methacrylate; 5, toluene diisocyanate (TDI).

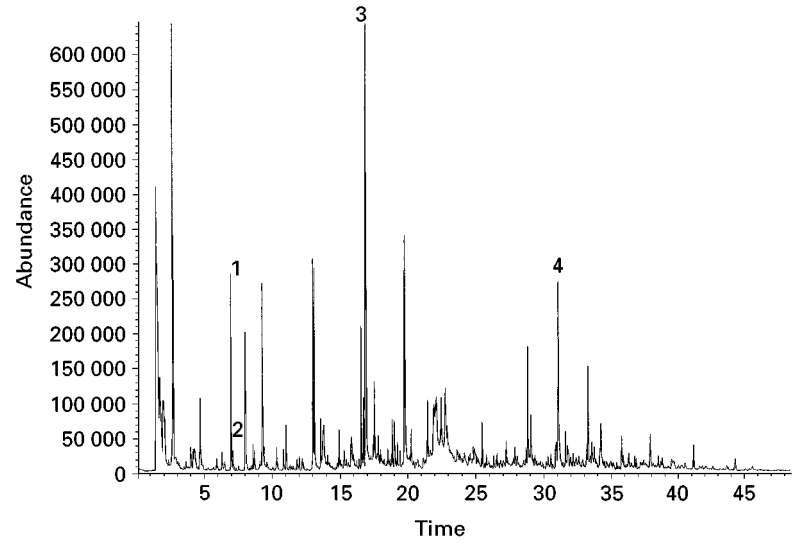

Figure 15 Read automobile paint with epoxy constituents. 1, Styrene; 2, butyl acrylate; 3, phthalic anhydride; 4, bis-phenol A (from epoxy).

the plasticizing agents into the polymer itself, instead of using an additive like a phthalate ester. Consequently, pyrograms of paints produced in the 1990s are likely to show no phthalate plasticizers, but to have peaks for long chain methacrylates instead. These methacrylates with long aliphatic substituents become part of the paint polymer, with the aliphatic tail providing flexibility in a way which will not be lost from the polymer by evaporation. **Figure 16** shows a pyrogram of a paint which used a substantial amount of styrene (1), made flexible by the addition of octyl methacrylate (2) which elutes at about 17 min, and is the largest peak in the chromatogram.

Paper Coatings

Papers are coated with a variety of polymeric materials to provide qualities such as gloss for magazines and waterproofing for food packaging, in addition to decorative and functional uses of inks and toners from printers and photocopiers. Although it is sometimes difficult to separate the coating from the paper fibres, this may not be required for many analyses. This is because the polymer coating and the paper will be pyrolysed essentially independently of each other, and the resulting pyrogram will contain information about each of the constituents. The pyrolysis products of cellulose are well documented, and consist of many oxygenated materials such as furans,

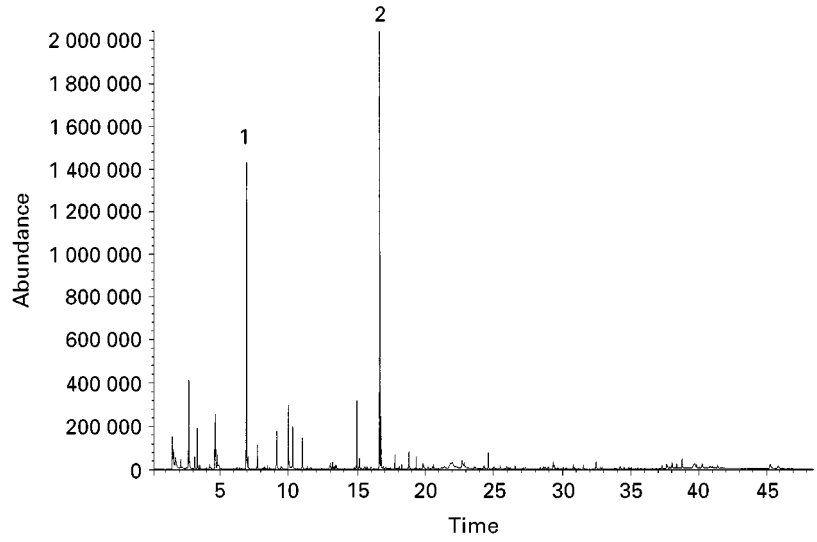

Figure 16 Automobile paint with long alkyl chain methacrylate. 1, Styrene; 2, octyl methacrylate.

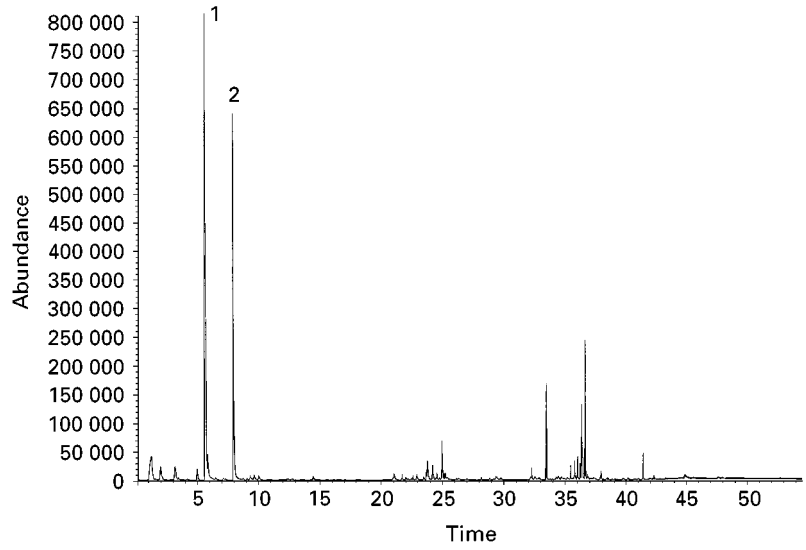

Figure 17 Coating for freezer carton. 1, Styrene; 2, α-methyl styrene.

plus levoglucosan, whereas the products from most coatings include monomers like styrene, acrylics and hydrocarbons, so differentiating them is generally straightforward. **Figure 17**, for example, shows the products of pyrolysing a coating used on cardboard boxes made for storing frozen foods. The two large peaks, eluting at about 6 and 8 min, are styrene and α-methylstyrene, respectively. When a piece of a box coated with this polymer is pyrolysed, the result is the pyrogram shown in **Figure 18**. Most of the peaks are cellulose pyrolysates, including furancarboxaldehyde (1), hydroxymethylfurancarboxaldehyde (4) and levoglucosan (5). The monomers styrene and α-methylstyrene, however, are superimposed on the cellulose products, and are seen as peaks 2 and 3. The furans, especially furancarboxaldehydes and levoglucosan, are reliable markers for cellulose and should be seen in any paper, coated or not.

In **Figure 19** they can be seen again, this time from a piece of glossy magazine paper. Since the paper is thinner than the cardboard box, the relative amount of the coating is larger here, and the polymer constituents forming the glossy finish show up readily. In this case, the coating is a copolymer of styrene, butadiene and methyl methacrylate, and each of the monomers represents one of the major peaks in the pyrogram.

Polyolefins are frequently used as coatings or laminates on to papers for a variety of purposes. The

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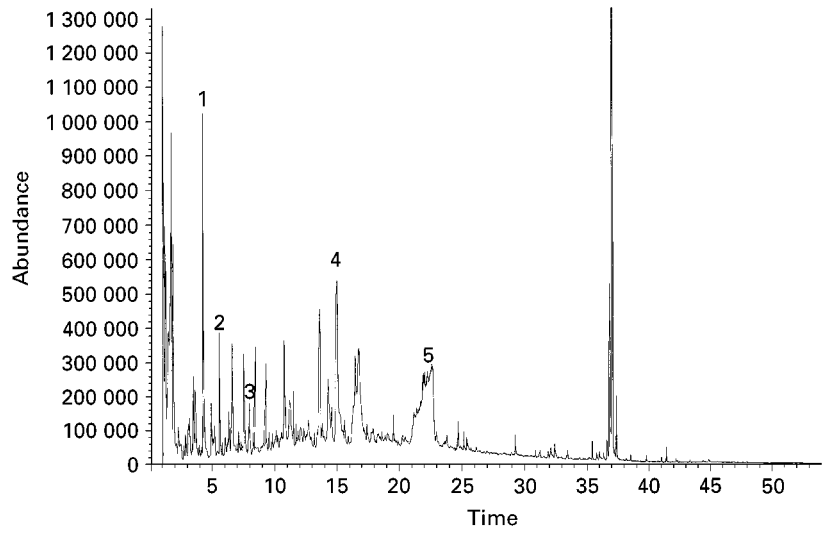

Figure 18 Pyrolysis of cardbord with coating shown in Figure 17. 1, Furancarboxaldhyde; 2, styrene; 3, α-methylstyrene; 4, hydroxymethylfurancarboxaldehyde; 5, levoglucosan.

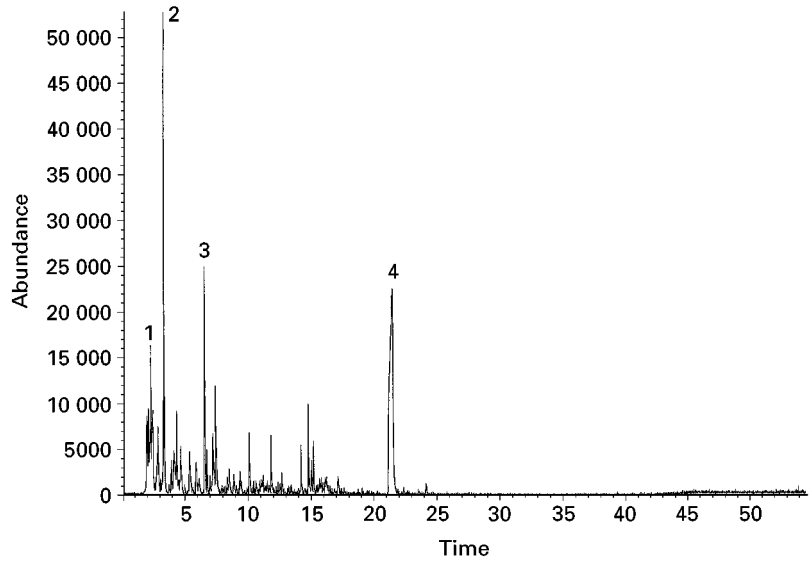

Figure 19 Pyrogram of a piece of glossy coated paper stock. 1, Butadiene; 2, methyl methacrylate; 3, styrene; 4, levoglucosan.

glossy coating on a photograph is likely to be polyethylene, as is the protective film on paper used as freezer-wrap. When polyethylene is pyrolysed, it is broken into many long chain hydrocarbons, including alkanes, alkenes and dienes. These polymer fragments elute in a series of triplet peaks, making a characteristic pattern for polyethylene. When a piece of paper coated with polyethylene is pyrolysed, the cellulose peaks are again seen, co-eluting with the polyethylene hydrocarbon fragments. **Figure 20** shows an example of this, being the pyrogram of a piece of coated freezer paper. Most of the larger peaks before 18 min are from cellulose, and the poorly shaped peak at 22 min is levoglucosan. All of the triplet peaks, including the one at 18 min and almost everything after 24 min, are normal hydrocarbons indicative of polyethylene pyrolysis.

Glass surfaces are also frequently covered with a coating, such as a silk screen ink for decorative pieces, a film for colouring or shading, or a layer to provide a working surface. **Figure 21** shows an interesting application of glass coating, in which laboratory glass slides were coated with a Teflon material to create wells for aqueous reagents. When Teflon is pyrolysed, it reverts almost entirely to monomer, tetrafluoroethylene. The coating which had been applied to the glass consisted largely of Teflon, plus a styrene/ethyl acrylate copolymer. When pyrolysed,

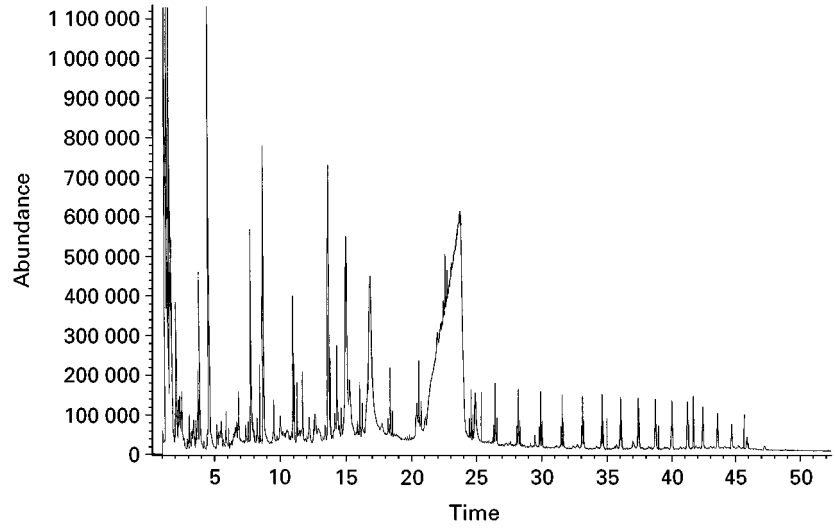

Figure 20 Pyrolysis of freezer paper (polyethylene-coated cellulose paper) at 750°C for 15 s.

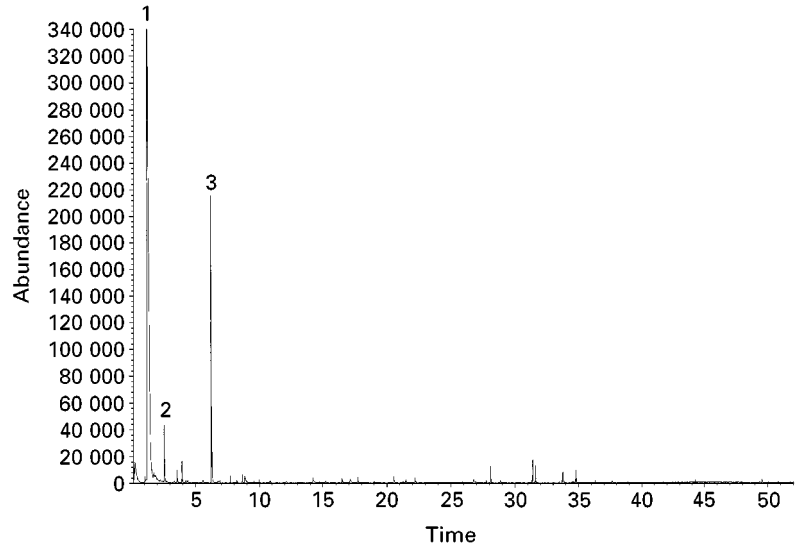

Figure 21 Coating applied to glass, pyrolysed at 750°C for 20 s. 1, Tetrafluoroethylene; 2, ethyl acrylate; 3, styrene.

a simple chromatogram results, revealing peaks for all three monomers, tetrafluoroethylene, ethylacrylate and styrene.

Future Developments

The development of more complex, specialty or high performance coatings will reinforce the need for detailed analysis in both quality control and product development laboratories. Pyrolysis-capillary GC-MS should become increasingly relied upon to unravel these complex polymer matrices. The development of fast GC techniques will do much to expand the use of this technique, since the time required to produce a well resolved pyrogram is perhaps the greatest drawback. The introduction of several automated systems for pyrolysis–GC-MS has already made the technique routine, enhancing reproducibility and efficiency of instrument use.

See also: **II/Chromatography: Gas:** Detectors: Mass Spectrometry; Pyrolysis Gas Chromatography. **III/Art Conservation: Use of Chromatography in.**

Further Reading

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